



Antibacterial Effect of *Juglans regia*, *Citrus sinensis*, *Vicia faba*, and *Urtica urens* Extracts under In vitro Conditions

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Abstract

Various agricultural products are known to have anti-microbial, anti-cancer, and anti-inflammatory effects. As we can mention, Walnut (*Juglans regia*) husk, orange (*Citrus sinensis*) peel, Broad bean (*Vicia faba*) peel, and Nettle (*Urtica urens*) are proven to have antimicrobial and anticancer actions. Also, plant diseases such as *Pectobacterium carotovorum*, *Ralstonia solanacearum*, *Dickeya chrysanthemi*, and *Pseudomonas syringae* are known to cause annual damage to plant products. Therefore, in the current study, the researchers evaluated these extracts' antibacterial activity on the mentioned bacteria under *in vitro* conditions. Extracts of *J. regia* husk, *C. sinensis* peel, *V. faba* outer peel and *U. urens* were prepared by maceration method and their anti-bacterial activity on *P. carotovorum*, *D. chrysanthemi*, *P. syringae*, *R. solanacearum* was evaluated using blank disk and well diffusion to obtain minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. *J. regia* (husk) and *V. faba* (outer peel) extracts revealed an anti-bacterial effect on all 4 studied bacteria, while *C. sinensis* (peel) only inhibited the growth of *P. syringae* and *U. urens* only had this effect on *P. syringae* and *R. Solanacearum*. The inhibition zones varied from 8 to 14 mm, while almost all MIC and MBC rates were 6.25 and 12.5 mg/mL, respectively. Based on the antimicrobial results, the extracts that showed suitable antibacterial effects on certain bacteria can be further studied to be used as natural pesticides.

Keywords: antibacterial effect, *in-vitro*, extracts

1. INTRODUCTION

Planting fruit trees is a worthwhile experience for both gardeners and commercial growers [1]. Anti-microbial effects of different extracts of agricultural products are truly valuable [2]. The genus Juglans includes numerous species which proved to have many benefits. Some extracts hold therapeutically active constituents, for example, polyphenols [3][4]. Walnut, *Juglans regia*, husk, a discarded property of walnut, is found to have a brown pigment called juglone, which is a cause of antimicrobial and anticancer activity [5]. Citrus is also known as one of the vital fruits. Orange, *Citrus sinensis*, is the most ordinary tree fruit in the world, which is grown in tropical and subtropical climates because of the sweet fruit acquired [6]. This very common fruit is proven to have adequate

antimicrobial effects [7]. Broad been peel, *Vicia faba* is also another common plant property. It has previously been proven to have some effects on the human body such as reduction of blood sugar and prevention of heart diseases. Its antimicrobial and anticancer effects have also been proven [8]. Based on the result of some studies, Nettle, *Urtica urens*, was found to have anti-inflammatory, anti-swelling, anti-oxidant, and anti-aging effects altogether [9].

Bacterial plant diseases are known to be the cause of a 10% loss in plant production [10]. Soft rot, a major plant disease is caused by *Pectobacterium carotovorum* (*P. carotovorum*) [11]. *P. carotovorum* is a main constraint in a wide range of vegetable and flower hosts [11]. As well, the Black leg is a severe disease with symptoms the same as soft rot and one of its major hosts is potato. The main responsible bacterium for this disease is *Dickeya chrysanthemi* [12]. Bacterial Wilt is also an epidemic harmful disease for Solanaceae vegetables, which lowers tomato yield by up to 91%. It is caused by a bacterium, called *Ralstonia solanacearum*. Current illness is known to have a sudden outbreak. It spreads quickly through water and soil throughout the agricultural land, and eventually contaminates most of the plants [13]. Both young and old fruit trees such as mango, apple, and hazelnut are at risk of getting bacterial canker, which causes major losses and is mainly

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Table 1. The average and standard deviations of bacterial inhibition zone using disk diffusion.

Microorganism	Inhibition Zone (mm)			
	<i>Juglans regia</i>	<i>Citrus sinensis</i>	<i>Vicia faba</i>	<i>Urtica urens</i>
<i>Dickeya chrysanthemi</i>	11.5±1.50	-	9.00±100	-
<i>Pectobacterium carotovorum</i>	10.3±0.47	-	11.0±0.00	-
<i>Pseudomonas syringae</i>	11.0±0.82	11.0±0.71	10.0±0.00	11.2±0.49
<i>Ralstonia solanacearum</i>	8.50±1.50	-	9.00±0.00	8.50±0.50

caused by *Pseudomonas syringae* [14].

Since *J. regia* husk, *C. sinensis* peel, *V. faba* outer peel, and *U. urens* have shown antibacterial effects on some other bacteria [1][3]-[9] and *P. carotovorum*, *D. chrysanthemi*, *R. solanacearum*, and *P. syringae* and are known to be a major pathogenic cause of some plant diseases [11]-[14]. In this study, we have evaluated their antibacterial activity under *in vitro* conditions.

2. MATERIALS AND METHODS

2.1. Microorganisms

Bacterial strains of *P. carotovorum*, *D. chrysanthemi*, *P. syringae*, and *R. solanacearum* were collected from the Iranian Research Institute of Plant Protection (IRIPP) and cultured on nutrient agar media under sterile conditions.

2.2. Plant Extracts

Extracts of *J. regia* husk, *C. sinensis* peel, *V. faba* outer peel, and *U. urens* were prepared by macerating 25 g of proper powder into 250 mL of 70% ethanol. The mixture was shaken for 24 h and made into 100 mg/mL concentrates while becoming sterilized by exposure to UV rays.

2.3. Antimicrobial assay

The effect of the ethanolic extracts was observed on the mentioned bacteria, using serial dilution and disc diffusion methods [15] on proper cultural media (nutrient agar/Muller-Hinton agar) and each time incubated at 25 °C for 24 h. This study was conducted 3 times. All of the process was done under the maximum possible sterile conditions.

2.3.1. Blank disk diffusion

To observe the Inhibition zone caused by these extracts 6.4 mm diameter blank disks were soaked

into the mentioned extract and each was put in the middle of nutrient agar media, in which studied bacteria were cultured a minute before. Then all the plates were incubated at 25 °C for 24 h.

2.3.2. Well diffusion

To observe the inhibition zone caused by these extracts, wells were created in the middle of nutrient agar media, in which studied bacteria were cultured a minute before. Then 20 µL of the mentioned extracts were poured into the wells and after that, all the plates were incubated at 25 °C for 24 h.

2.3.3. Minimum inhibitory concentration (MIC)

The MIC of the extracts mentioned was determined by culturing the bacteria in Muller-Hinton broth [16]. The culture was standardized by using a standard microbiological 0.5 McFarland standard tube. Possible antimicrobial agents were each serially diluted separately 5 times, 1:1 through Muller-Hinton Broth as a sterile diluent. After the antimicrobial agents were diluted, a volume of standardized inoculum for bacteria was added to each dilution tube (equal to the volume of diluted agent), and a positive test control tube was included to prove microbial growth over the course of incubation and the sterility of media. The tubes, containing microorganisms and antimicrobial agents, were then incubated at 25 °C for 24 h. Because of the tint of antimicrobial agents used, no turbidity or pellet of microorganisms was distinguishable from the media, and therefore all the serially diluted tubes' containing's were cultured in Muller-Hinton agar media and incubated at 25 °C for 24 h. After the incubation, the cultured Muller-Hinton agar plates were observed for microbial growth. The one with less concentrated antimicrobial agent than the last plate in the series, which did not demonstrate growth (MBC), showed

less growth and paralleled with the MIC of the antimicrobial agents.

2.3.4. Minimum Bactericidal Concentration (MBC)

The MBC of the antimicrobial agent was determined by culturing the bacteria in Muller-Hinton broth of 0.5 McFarland standard [16]. Also, antimicrobial agents were each serially diluted using 1:1 Muller-Hinton Broth as a sterile diluent and inoculated with an equal volume of microorganisms, including a positive test control tube to prove microbial growth over the course of incubation and the sterility of media. Then the tubes were incubated at 25 °C for 24 h. All the diluted tubes containing the most antimicrobial agents concentrated to the least, were cultured in Muller-Hinton agar media and incubated at 25 °C for 24 h. After the incubation, the cultured Muller-Hinton agar plates were observed for microbial growth. The last plate in the series that did not demonstrate growth linked with the MBC of antimicrobial agent.

3. RESULTS AND DISCUSSIONS

3.1. Disk and well diffusion

Based on the results mentioned in Tables 1 and 2, *J. regia* (husk) and *V. faba* (outer peel) extracts revealed anti-bacterial effect on all four studied bacteria, while *C. sinensis* (peel) only inhibited the growth of *P. syringae* and *U. urens* only had this effect on *P. syringae* and *R. solancearum*.

3.2. The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

As expected, the antibacterial effects of plant extracts on the studied pathogens were the same as diffusion methods (Table 3). Considering MIC and MBC values brought, the efficiency of plant extracts were similar except the fact that effectiveness of *V. faba* (outer peel) on *P. carotovorum* and *U. urens* on

R. solancearum was proven to be less.

3.3. Discussion

This experiment aims to control or treat plant diseases such as soft rot and bacterial wilt. Antibiotics and fungi pesticides such as captan, streptomycin, difenoconazole and streptocycline are applied to prevent or treat diseases caused by *P. carotovorum*, *R. solanacearum*, *D. chrysanthemi* and *P. syringae* pathogens, respectively [17]-[20]. However, anti-biotic resistance has become a worldwide environmental challenge and also some anti-biotics are also known to have undesirable effects on chloroplast and nuclear gene expression [21][22]. Additionally, fungicides such as captan itself are known to have harmful consequences on soil microbial activity [23]. Owing to all these environmental damages, plant extracts are now becoming an alternative to these pesticides [24].

Respectively, based on the previous publications on *C. sinensis*, *J. regia*, *V. faba* and *U. urens*, the authors declared their antibacterial activity on some other bacteria [1][6][8][9]. In the current study, their effect has been studied on major plant pathogens *D. chrysanthemi*, *P. carotovorum*, *P. syringae*, and *R. solancearum*, which annually cause great harm to a vast range of plant hosts [11]-[14].

Based on findings of current study, *C. sinensis* (peel) only had antibacterial effect on *P. syringae*. Considering the inhibition zone, showing 8 ± 0 mm in diameter, the extract had a lower antibacterial effect compared to *E. coli*, while had a higher one on *P. aeruginosa*, the inhibition zones respectively being 12.6 ± 0.94 and 4.33 ± 1.24 mm [25]. When the MIC and MBC records were compared, its inhibiting and killing effects were respectively same and higher on some other bacteria such as *S. typhi* and *S. aureus* [26]. Extracts of *V. faba* (outer peel) and *J. regia* (husk) both had antimicrobial

Table 2. The average and standard deviations of bacterial inhibition zone using agar well diffusion.

Microorganism	Inhibition Zone (mm)			
	<i>Juglans regia</i>	<i>Citrus sinensis</i>	<i>Vicia faba</i>	<i>Urtica urens</i>
<i>Dickeya chrysanthemi</i>	11.5 ± 1.50	-	9.00 ± 100	-
<i>Pectobacterium carotovorum</i>	10.3 ± 0.47	-	11.0 ± 0.00	-
<i>Pseudomonas syringae</i>	11.0 ± 0.82	11.0 ± 0.71	10.0 ± 0.00	11.2 ± 0.49
<i>Ralstonia solancearum</i>	8.50 ± 1.50	-	9.00 ± 0.00	8.50 ± 0.50

Table 3. MIC and MBC values of the four plant extracts on the studied bacteria.

Microorganism	Minimum Inhibitory Concentration (mg/mL)			
	<i>Juglans regia</i>	<i>Citrus sinensis</i>	<i>Vicia faba</i>	<i>Urtica urens</i>
<i>Dickeya chrysanthemi</i>	6.25	-	6.25	-
<i>Pectobacterium carotovorum</i>	6.25	-	12.5	-
<i>Pseudomonas syringae</i>	6.25	6.25	6.25	6.25
<i>Ralstonia solancearum</i>	6.25	-	6.25	12.5
Microorganism	Minimum Bactericidal Concentration (mg/mL)			
	<i>Juglans regia</i>	<i>Citrus sinensis</i>	<i>Vicia faba</i>	<i>Urtica urens</i>
<i>Dickeya chrysanthemi</i>	12.5	-	12.5	-
<i>Pectobacterium carotovorum</i>	12.5	-	25.0	-
<i>Pseudomonas syringae</i>	12.5	12.5	12.5	12.5
<i>Ralstonia solancearum</i>	12.5	-	12.5	25.0

effect on all studied bacteria, while the greatest rate was on *P. syringae* with an inhibition zone of 10 ± 0 and 14 ± 0 mm, respectively. In comparison to some Gram-positive bacteria such as *S. agalactiae*, *V. faba* (outer peel) extract showed less inhibiting and bactericidal effects. Overall, considering current and other studies, even though both extracts were found to have suitable antimicrobial effect, whereas *J. regia* (husk) seemed to be more effectual than *V. faba* (outer peel) [27]. Based on the reported inhibition diameter, MIC and MBC rates for *J. regia* (husk) suggest its higher effectiveness even compared with other bacteria, such as *B. cereus*, *P. mirabilis* and so on [28][29]. Unlike the two mentioned plant extracts, *U. urens* only showed antibacterial effect on *P. syringae* and *R. solancearum*. Considering other studies, these inhibition zones and bactericidal effects are considered more than acceptable [30][31].

Bioactive compounds in plants such as flavonoids and coumarins are known to be the cause of many features like anti-bacterial, antioxidant, and anti-cancer activities [32]. Since, α -pinene, juglone, quercetin and gallic acid have respectively been reported as major bio-active constituents of *C. sinensis*, *J. regia*, *V. faba*, and *U. urens*, it can be guessed that these compounds are the cause of antimicrobial activity of the studied plant extracts on *D. chrysanthemi*, *P. carotovorum*, *P. syringae*, and *R. solancearum* and even other bacteria [1][33]-[35].

4. CONCLUSIONS

The current study proved the antibacterial activity of *Juglans regia* and *Vicia faba* on all the studied bacteria, *Citrus sinensis*' activity on *Pseudomonas syringae* and *Urtica urens* activity on *Pseudomonas syringae* and *Ralstonia solancearum*. Since the results were mostly fortunate, these extracts have the potency to be used as the natural pesticide against the bacteria they showed antimicrobial effect on. It would be proper for the current study to be further analyzed under *in-vivo* and *in-silico* conditions to reconfirm this potency and discover the procedure needed.

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